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A new species of *Cincticostella* (Ephemeroptera, Ephemerellidae) from China

Chonlakran Auychinda[1], Dávid Murányi[2,3], Weihai Li[4], Michel Sartori[5,6], Jean-Luc Gattolliat[5,6]

1 *Animal Systematics and Ecology Speciality Research Unit (ASESRU), Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 1090, Thailand*
2 *Department of Zoology, Eszterházy Károly University, Leányka u. 6, H-3300 Eger, Hungary*
3 *Department of Zoology, Hungarian Natural History Museum, Baross u. 13, H-1088 Budapest, Hungary*
4 *Department of Plant Protection, Henan Institute of Science and Technology, Xinxinag, Henan 453003, China*
5 *Museum of Zoology, Palais de Rumine, Place Riponne 6, CH-1005 Lausanne, Switzerland*
6 *University of Lausanne (UNIL), Department of Ecology and Evolution, CH-1015 Lausanne, Switzerland*

http://zoobank.org/5C92D79F-984A-4465-844E-C0E08B41E903

Corresponding author: Jean-Luc Gattolliat (jean-luc.gattolliat@vd.ch)

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Abstract

Cincticostella tornata, a new species of Ephemerellidae, is described from China. It belongs to the *insolta*-species group and is closely related to *C. femorata*. It is hitherto only known from its type locality in central China, more than 1000 km northern to the known distribution of *C. femorata*. In addition, the *COI* barcode of the new species is provided.

Key Words

Cincticostella femorata, *Cincticostella tornata*, *COI*, mayflies

Introduction

Cincticostella was established by Allen (1971) as a subgenus of *Ephemerella* Walsh, 1862, then raised to the generic level the following year (Tshernova 1972). It currently encompasses 17 valid species (Kluge 2019). They are distributed in eastern Palearctic and Oriental regions, including China, India, Japan, Nepal, Thailand and Vietnam. In China, nine species were until now reported: *C. colossa* Kang & Yang, 1995, *C. elongatula* (McLachlan, 1875), *C. femorata* (Tshernova, 1972), *C. fusca* Kang & Yang, 1995, *C. gosei* (Allen, 1975), *C. insolta* (Allen, 1971), *C. levanidovae* (Tshernova, 1952), *C. nigra* (Ueno, 1928), *C. orientalis* (Tshernova, 1952)) (Xie et al. 2009). The larvae can be identified by having the pronotum with a prominent anterolateral projection, the mesothorax with a pair of large wide anterolateral processes, abdominal tergites with paired dorsal tubercules and lamellate imbricate gills on segments III to VII (Allen 1971; 1975). Allen (1975) divided *Cincticostella* in two species groups: the *insolta*-group and the *nigra*-group. The *insolta*-group is characterized by suboccipital head tubercles and the mid and hindfemora enlarged with characterized protuberances, so-called chalazae (Jacobus and McCafferty 2008). A new subgenus of *Cincticostella*, *Rhionella* Allen, 1980, was subsequently established for this species-group (Allen 1980). Jacobus and McCafferty (2008) recognized the monophyly of this species-group, but they considered *Rhionella* as strict synonym of *Cinsticostella* to avoid rendering the rest of *Cincticostella* paraphyletic. In his non-ranking phylogeny of Ephemeroptera, Kluge (2004) still considered *Rhionella* as valid and *Cincticostella* as a "plesiomorphon", i.e. a taxon only defined by plesiomorphies.

Cincticostella femorata (Tshernova, 1972) was originally described from Vietnam based on a single larva. It was assigned to *Asiatella* Tshernova, 1972, but was transferred to *Cincticostella* as addendum in the same article (Tshernova 1972). This species appears highly de-

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rived within the *insolta*-group as the mid and hind femora are greatly flattened with strong chalazae on dorsal and sometimes ventral margins, and the prothorax is much broader than in related species. A closely related species, *Cincticostella boja* (Allen, 1975) was subsequently established for larvae from Thailand, described and figured but unnamed by Gose (1969). The description was just translated from Gose (1969), and no material was examined by Allen (1975). He considered that *C. boja* was significantly different from other species and especially from *C. femorata* by the quadrangular head, the presence of paired submedian tubercles on tergites II to X and the well-developed posterolateral projections on segments VII to IX (Allen 1975). Jacobus et al. (2005) examined five larvae from three different Thai localities. They compared the main morphological characters used by Allen (1975), as well as the number of denticles on claws and the relative density of hair-like setae on the caudal filaments and concluded that all specimens present important but intraspecific variations. They consequently considered *C. boja* as a junior synonym of *C. femorata* (Jacobus et al. 2005). Comparisons of original illustrations and descriptions of the two species as well as more recent illustrations of specimens attributed to *C. femorata* from China (Xie et al. 2009) and Thailand (Martynov et al. 2019) challenged this synonymy. The species is now considered as occurring in Vietnam, Thailand and China (Martynov et al. 2019; Xie et al. 2009).

We had the opportunity to examine material recently collected in China by two of us (DM and WL). Despite important similarities with *C. femorata*, part of the characters did not match the original and subsequent descriptions of the species. We therefore consider that this material belongs to a new species. The description of this new species may help to better understand the intra- and interspecific variability within *C. femorata*; in the future *C. femorata* may appear as a complex of closely related species.

Material and methods

Collecting

The specimens were collected with small sized aquatic net, during a field trip primarily focused on collecting Plecoptera. They were fixed in 75% ethanol on the field. Holotype and paratypes are kept in the MZL (Museum of Zoology, Lausanne, Switzerland), one paratype in the HNHM (Hungarian Natural History Museum, Budapest, Hungary), one paratype in HIST (Henan Institute of Science and Technology) as indicated in the text.

Morphological examination

All dissected specimens were entirely mounted on slides in Euparal medium. Drawings and pictures of body parts were made using an Olympus BX51 stereoscopic microscope with a camera lucida or a digital camera Olympus SC50. Extended depth of focus images were obtained using the software Stream Basic 1.9.4. Pictures of the whole larval body were taken with the Visionary LK system (Dun., Inc., USA). Pictures and drawings were subsequently enhanced with Adobe Photoshop CC2015.

Genetics

DNA of part of the specimens was extracted using non-destructive methods allowing subsequent morphological analysis (see Vuataz et al. 2011 for details). We amplified a 658 bp fragment of the mitochondrial gene cytochrome oxidase subunit 1 (COI) using the primers LCO 1490 (GGTCAACAAATCATAAAGATATTGG) and HCO 2198 (TAAACTTCAGGGTGACCAAAAAAT-CA) (Folmer et al. 1994). The polymerase chain reaction was conducted with an initial denaturation temperature of 98 °C for 30 sec followed by a total of 37 cycles with denaturation temperature of 98 °C for 10 sec, an annealing temperature of 50 °C for 30 sec and an extension at 72 °C for 30 sec, final extension at 72 °C for 2 min. Sequencing was done with Sanger's method (Vuataz et al. 2011). The genetic variability between specimens was estimated using Kimura-2-parameter distances (K2P), calculated with the program MEGA 7 (Kumar et al. 2016).

Results

***Cincticostella tornata* Auychinda & Gattolliat, sp. nov.**

http://zoobank.org/8230C5FE-CB09-4C40-AAD6-E163C6AC9986

Material. ***Holotype:*** China • Larva; Shaanxi, Hanzhong city, Foping county, Qinling Mts, slow forest brook in Panda valley; Alt. 1330m. 33°40.368'N, 107°58.327'E; 20 Apr. 2018; Coll. W.H. Li, R.R. Mo, D. Murányi; MZL GBIFCH 00763636.

Paratypes: China • 5 larvae; same data as for holotype; 2L in alcohol GBIFCH 00673084; 1L on slide GBIFCH 00606852; 1L on slide used for DNA extraction GBIFCH 00654874 all deposited in MZL. 1L in alcohol deposited in HNHM. 1L in alcohol deposited in HIST.

Description. ***Larva:*** Body length 8.7–10.0 mm; caudal filaments length 8.0–9.0 mm. Body yellowish-brown.

Head: Covered with thin setae. One pair of small, rounded protuberances between eyes. Genae quadrangular, well developed, angles rounded (Fig. 1). Antennae with scapus short, pedicellus as broad as long, flagellum of about 25 segments, first segment as long as next three segments.

Mouthparts: Labrum wide, apicolateral angle rounded; apicomedial emargination shallow, dorsal surface densely covered with long, hair-like setae and long stout flattened setae scattered over surface (Fig. 2A); ventral surface with numerous, long, stout, hair-like setae laterally orientated; apical margin with numerous feathered setae and very thin hair-like setae (Fig. 2B). Mandibles with numerous, hair-like setae on 2/3 proximal of dorsal and lateral surfaces (Figs 2C, D). Left mandible: outer incisor composed of

Figure 1. *Cincticostella tornata*: **A** habitus in dorsal view; **B** habitus in ventral view. Scale bar: 1 mm.

three teeth, two outer teeth stout, broad, apically rounded and one small, blunt tooth; inner incisor with one main stout, apically pointed tooth and one inner vestigial tooth; stout rectangular prostheca with bunch of hair-like setae on inner side (Fig. 2E). Right mandible: outer incisor composed of two spoon-shaped teeth, outer tooth longer

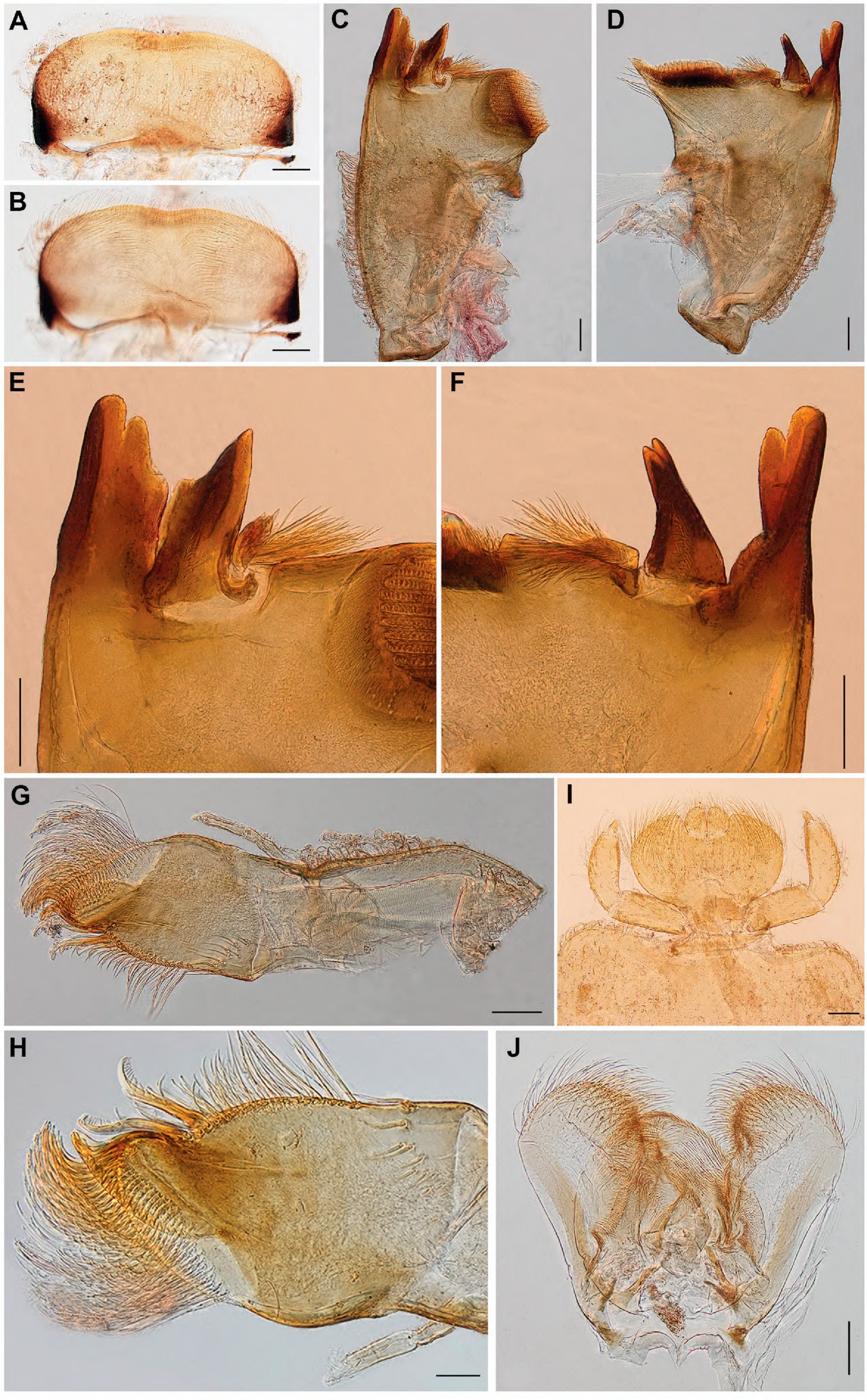

Figure 2. *Cincticostella tornata*: **A** dorsal view of labrum; **B** ventral view of labrum; **C** left mandible; **D** right mandible; **E** left mandibular canine; **F** right mandibular canine; **G** maxilla; **H** crown of maxilla; **I** labium; **J** hypopharynx. Scale bars: 0.1 mm (A–D, G–I, J); 0.05 mm (E, F, H).

than inner; inner incisor composed of two apically pointed teeth, orientated perpendicularly to outer incisor; prostheca consisting of numerous hair-like setae (Fig. 2F); eight hair-like setae on margin below mola (Fig. 2D). Maxilla: apex with transformed ventral canine typical of genus (Kluge 2004); two dentisetae with serrated inner margin; apex of maxilla surface with numerous, long, stout, hair-like setae, some with serrated inner margin; inner margin of galealacinia with 3–4 rows of long, stout, simple setae; 10–12 feathered, stout setae roughly arranged in a row near base of galealacinia surface (Fig. 2G, H). Maxillary palp: three segmented; segments partially fused; segment I slightly longer than half of segments II and III combined; segment III short, pointed, with few thin setae on apex (Fig. 2G, H). Labium with glossae semicircular, almost two times longer than broad; ventral surface of glossae covered with long, stout, simple setae; dorsal surface with an arc of long simple setae parallel to outer margin; glossae protruding over apices of paraglossae; paraglossae semicircular, dorsal surface covered with numerous long stout simple setae, ventral surface with flame-like setae. Ventral surface of mentum and submentum mostly covered with flame-like setae. Labial palp three-segmented; segments I and II equal in length, covered with long, hair-like setae; segment II crescent shaped, outer margin with spine-like long setae and long hair-like setae, inner margin covered with numerous long thin setae; segment III conical, two times longer than broad at base, apex covered with short thin setae (Fig. 2I). Hypopharynx: surface of lingua covered with short setae, most abundant in apical part; apices of superlinguae rounded with numerous hair-like setae (Fig. 2J).

Thorax: dorsal surface covered with scattered small thin setae. Pronotum: broad projection anterolaterally enclosing head, margin rounded except concave apically (black arrow tip on Fig. 3A); projections covered with long hair-like setae; laterally with short flame-like setae. Mesonotum: broad, large, rounded anterolateral projection, covered with long hair-like setae; laterally with short flame-like setae; small pair of triangular protuberances between forewing pads (white arrow tips on Fig. 3A). Forelegs: moderately expanded; length ratio of femur : tibia : tarsus = 2.9 : 2.4 : 1. Forefemora: dorsal surfaces cover with long hair-like setae; dorsal margin slightly concave, with dense setae and with only three blunt chalazae near distal angle; ventral margin with dense setae without serration; apex with one ventral and one dorsal well-developed extension (Fig. 4A). Foretibiae completely covered with long hair-like setae and short stout setae, apex of ventral margin produced and covered with a bunch of dense, long, thin setae. Foretarsi covered with long hair-like setae, ventral margin with stouter longer feathered setae. Foreclaws hooked with a row of four triangular teeth, two proximal teeth closer to each others (Fig. 4D). Mid legs: greatly expanded; length ratio of femur : tibia : tarsus = 3 : 2.7 : 1. Mid femora: dorsal surface cover with hair-like setae; ventral margin with dense setae and four serrated chalazae in proximal half of femora, ending with a well-developed triangular pointed process; dorsal margin with dense setae and eight chalazae progressively larger (Fig. 4B). Mid tibiae similar to foretibiae except setae less abundant and short flame-like setae on dorsal margin and apically. Mid tarsi: ventral margin with setae not feathered. Mid claws similar to foreclaws. Hind legs: greatly expanded; length ratio of femur : tibia : tarsus = 4.2 : 3.2 : 1; hind femora similar to mid femora except chalazae of ventral and dorsal margins: ventral margin with three chalazae medially; dorsal margin with 12 chalazae along margin and progressively larger (Fig. 4C). Hind tibiae and hind tarsi similar to mid tibiae and mid tarsi, respectively. Hind claws similar to foreclaws.

Abdomen: surface of tergites covered with hair-like setae and few stout flame-like setae, absent laterally on tergites IV to IX; distal margin smooth without spines. Tergites I–X with a pair of tubercles, minute on tergites I–III and X (Fig. 4E); highly developed on tergites IV–IX (Fig. 3B): arched, acute tubercles covered with medium setae (Fig. 3C). Sternites densely covered with medium setae; distal margin smooth without spines. Triangular, slender distolateral expansions present on abdominal segments I to IX, highly developed on segments IV to IX, covered with dense flat medium and hair-like setae. Gills present on segments III to VII (Fig. 3E–I); upper lamellae dorsally densely covered with flame-like setae, upper lamellae of gills III to V subrectangular, of gills VI roughly paddle-like, of gills VII suboval and reduced; lower lamellae bifurcate with marginal processes, bilobed on gills III to V, simple on gills VI and VII. Paracercus subequal in length to cerci; cerci and paracercus densely covered with hair-like setae, apex of each segment with very long, flattened setae (Fig. 3D).

Adults. Unknown.

Etymology. The Latin word "*tornata*" means rounded, in reference to the genae and anterolateral projection of mesonotum.

Diagnosis. *Cincticostella tornata* sp. nov. differs from closely related species of the *insolta*-group by the following combination of characters: 1) glossae protruded beyond apices of paraglossae; 2) genae extended with round apex; 3) anterolateral projection of pronotum pointed with a concave margin; 4) mesothorax with broad, rounded proximolateral expansion; 5) number and degree of development of chalazae on ventral and dorsal margins of mid and hind femora; 6) degree of development of apicoventral projection on ventral margin of fore-, mid and hind femora; 7) apicolateral projection of tergite IX well developed exceeding 1/3 of tergite X.

Distribution. The new species is only known from the type locality located in the Qinling mountains (Shaanxi province, close to Hanzhong city). This mountain range is considered as a natural boundary between South and North China and between the Palaearctic and Oriental realms (Fig. 5). A huge diversity of plant and animals is considered as endemic from this area, among others a subspecies of the giant panda (*Ailuropoda melanoleuca qinlingensis*) and the golden snub-nosed monkey

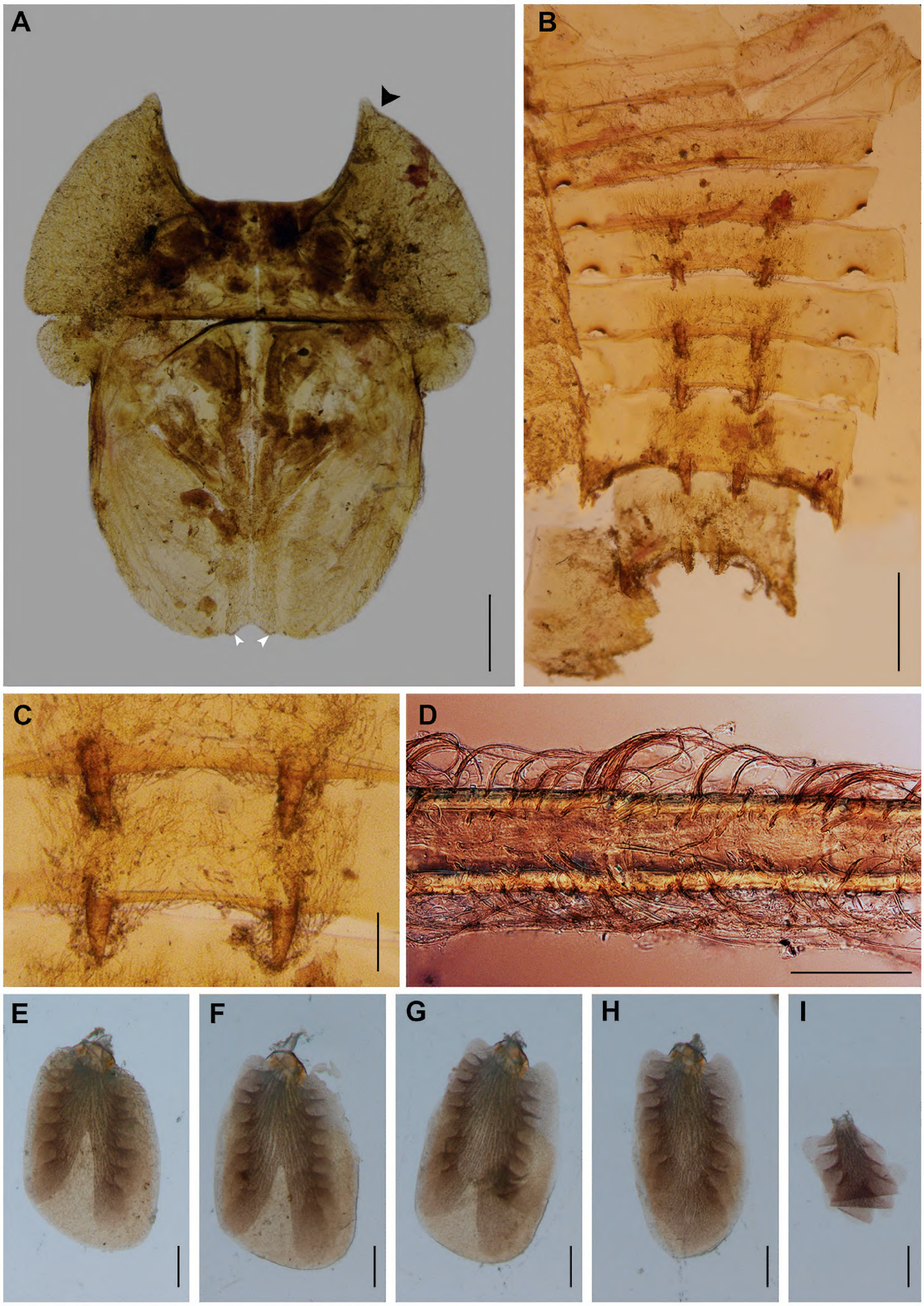

Figure 3. *Cincticostella tornata*: **A** thorax (black arrow tip: concave apical margin of prothorax; white arrow tips: small pair of triangular protuberances between forewing pads); **B** abdomen; **C** tergites VI–VII; **D** cercus; **E–I** gills of segments III–VII respectively. Scale bars: 1 mm (A, B); 0.25 mm (C–I).

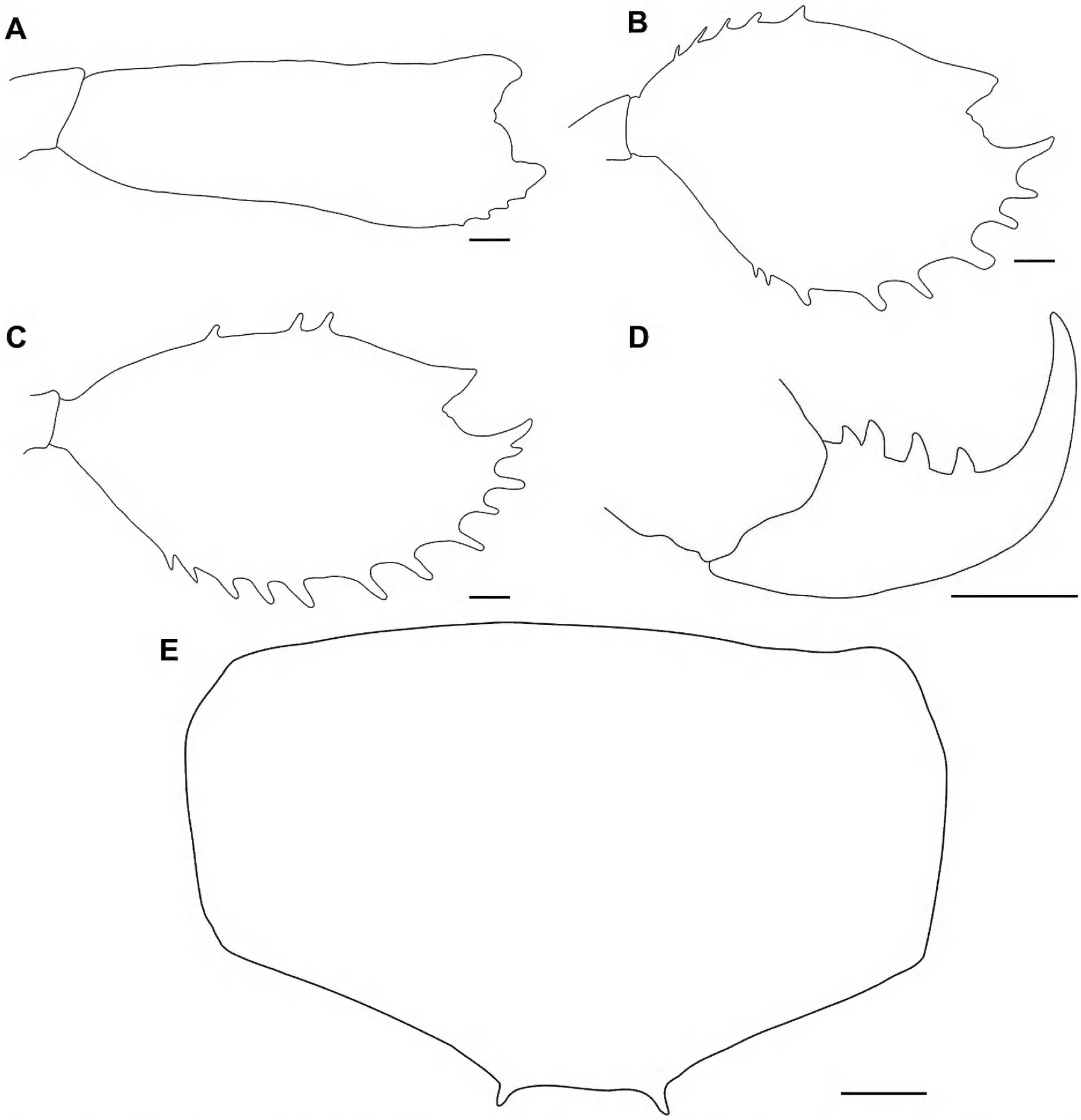

Figure 4. *Cincticostella tornata*: **A** forefemur; **B** mid femur; **C** hind femur; **D** forefemur claw; **E** tergite X. Scale bars: 0.2 mm (A–C); 0.1 mm (D, E).

(*Rhinopithecus roxellana*). At our present state of knowledge, it is not possible to know if *Cincticostella tornata* sp. nov. is endemic to this mountain range or is more widely distributed through China.

Habitat. The larvae were found in a small forest brook of relatively slow current. Maximum width of the brook is about 1.5 meter; depth varies between 10 and 30 centimetres. The substrate is mostly stony and sandy, mixed with silty patches and a moderate amount of debris. Accompanying fauna included two Holarctic genera of mayflies, *Baetis* (Baetidae) and *Ameletus* (Ameletidae), as well as the stoneflies *Rhopalopsole basinigra* Yang & Yang, 1995, *Spaeronemoura grandicauda* (Wu, 1973), *Nemoura* sp., and two species recently described from the same area, namely *Amphinemura albicauda* Li, Mo, Dong, Yang & Murányi, 2018 and *Amphinemura dingoidea* Li, Mo, Dong, Yang & Murányi, 2018 (Li et al. 2018).

Molecular results. A sequence of 658 bp, corresponding to the usual fragment of the *COI* gene, was obtained. The sequence was deposited in GenBank (Accession number: MT254050). It was compared to other known sequences found in databases such as GenBank and BOLD. The closest sequence corresponds to a North America Ephemerellidae (*Drunella walker* (Eaton, 1884)) with less than 83% of identity. Around twenty sequences were found with a percentage of identity comprising between 80 and 83, most of them belonging to the families Ephemerellidae and Caenidae. K2P distance

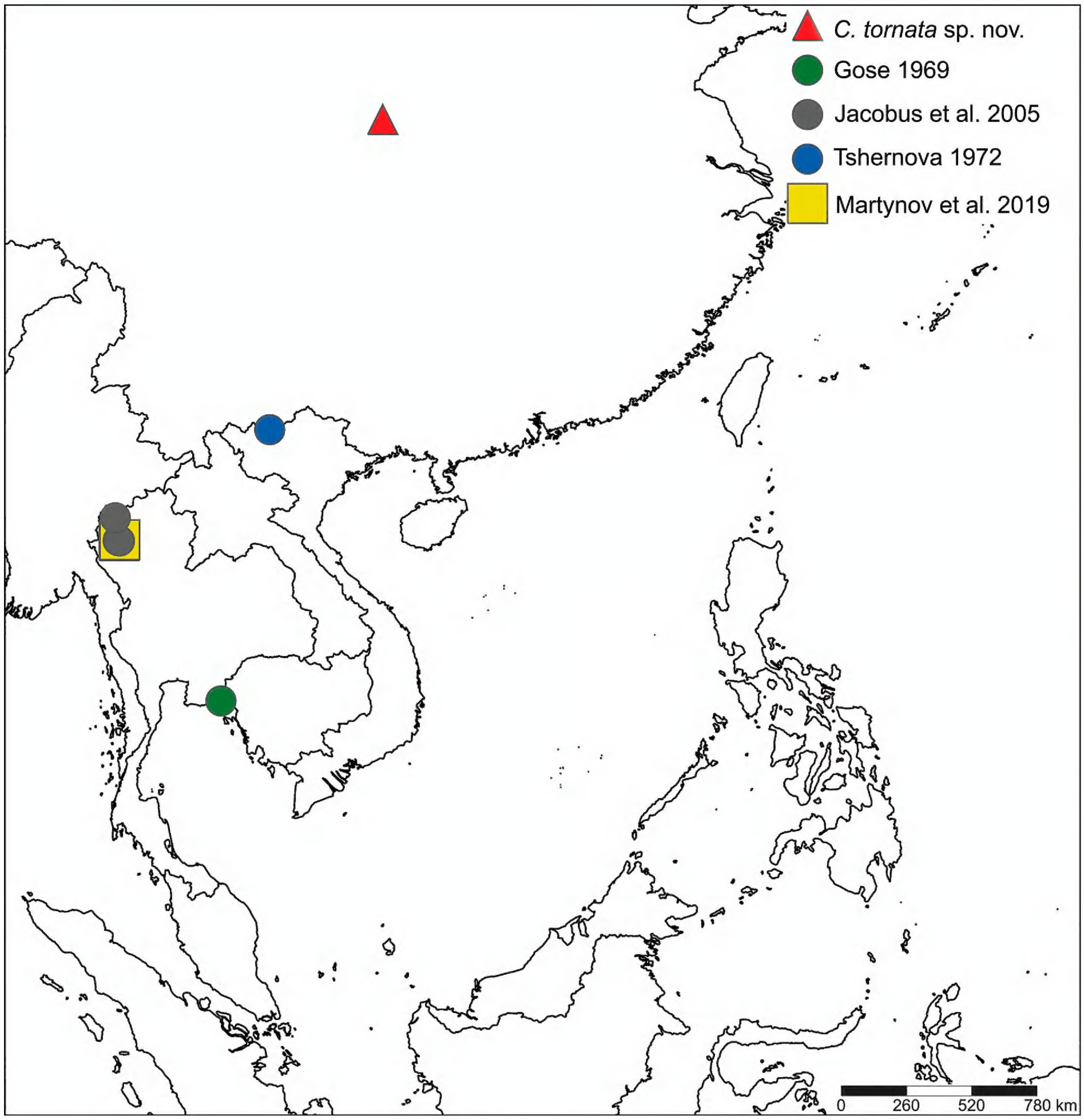

Figure 5. Map distribution of *C. femorata* and *C. tornata*. *C. femorata* represented by green circle (Gose 1969), blue circle (Tshernova 1972), gray circle (Jacobus 2005) and yellow square (Martynov et al. 2019); *C. tornata* represented by red triangular. Localities of *C. femorata* in China are not reported on the map as the species was mentioned from China by Xie et al. 2009 but without further information.

was also calculated with a sequence of *Cincticostella gosei* from Thailand (collected and sequenced by the first author, GenBank accession number: MN186574.1). The distance between the two species was estimated to 21.6% (meaning only 78.4% of identity).

Discussion

Within the *insolta*-group, *C. femorata* and *C. tornata* share two presumably derived characters, namely the anterolateral expanded prothorax and the extremely flattened mid and hind femora. The degree of development, the position and the orientation of the tubercles on the abdominal tergites of *C. tornata* are different from other species of the group. However, these characters must be considered with caution as it was recently demonstrated in a species of *Notacanthella* Jacobus and McCafferty, 2008. Two morphotypes were present based on the shapes of the tubercles, but these intraspecific variabilities mainly correspond to different ontogenic stages (earlier stages having more prominent tubercles)

Table 1. Larval character comparisons of *C. femorata* and *C. tornata*.

Characters	*C. femorata* (Tshernova 1972)	*C. femorata* (Martynov 2019)	*C. femorata* = *C. boja* (Gose 1969)	*Cincticostella tornata* sp. nov.
Labium	Glossae not protruding beyond paraglossae	–	Glossae not protruding beyond paraglossae	Glossae protruding beyond paraglossae
Maxillary canine	Well-developed, sharp, conical	–	Reduced, length less than width	Reduced, length less than width
Chalazae: dorsal/ ventral margin of forefemora	3/none	3/none	None/none	3/none
Chalazae: dorsal/ ventral margin of mid femur	4/none	9/none	7/3	9/6
Chalazae: dorsal/ ventral margin of hind femur	11/none	9/none	11/5	12/4
Anterolateral projection of pronotum	Broad, apically blunt, convex below tip	Broad, apically blunt, convex below tip	Broad, apically pointed, concave below tip	Broad, apically pointed, concave below tip
Claws	Somewhat hooked, 2 small teeth	–	Somewhat hooked, 3–4 small teeth	Hooked, 4 teeth
Genae	Extended with a rounded apex	Extended with a rounded apex	Extended, with a little corner	Extended with a rounded apex
Prostheca right mandible	–	–	Well-developed with a bunch of simple setae	Reduced with a bunch of simple setae
Prostheca left mandible	Well-developed, conical with a bunch of simple setae	–	Well-developed, slender with a bunch of simple setae	Well-developed, rounded with a bunch of simple setae
Posterolateral projection of abdominal tergites	IV–IX	III–X	II–X	I–X
Lateral projection of tergite IX	Moderately developed, not extended beyond tergite X	Well-developed, extended beyond tergite X	Well-developed extended equally tergite X	Well-developed extended beyond tergite X
Cerci	High density of long hair-like setae	–	Low density of short hair-like setae	Low density of medium hair-like setae
Small occipital spines on head	Present	–	Present	Present
Distribution	Vietnam	Thailand	Thailand	China

(Auychinda et al. 2020). As already mentioned, *C. femorata* and *C. tornata* only differ by minute characters which may be easily overlooked. In particular, when using the keys to identify species of *Cincticostella* (Allen 1975; Xie et al. 2009), one will arrive to *C. femorata* without discrepancies. This easily explains why all the larvae with expanded prothorax, flattened femora and developed genae were previously identified as *C. femorata*. A global revision involving all available material from South East Asia is highly recommended for the species delimitation of *C. femorata*, including the confirmation or rejection of the synonymy of *C. boja* with *C. femorata* as well as the correct attribution of the material from South and Central China to one of these species. This revision should be ideally based on integrative taxonomy, especially by using both morphological and molecular evidences. The comparison of the characters in the closely related species is summarized in Table 1. Noteworthy, the imagos all these taxa remain unknown. The recent discovery and description of imaginal *C. fusca* revealed that male genitalia (penes and gonopods), tergite pattern and pigmentation of hind wings are reliable characters to distinguish the different species of *Cincticostella* (Zhang et al. 2020). We may expect that imaginal characters will confirm the separation and validity of these closely related species.

The type-locality of *C. tornata* is located 1200 km north to those of *C. femorata* and 2350 km north to those of *C. boja* (1700 km north to the closest report from Thailand) (Martynov et al. 2019). These long distances may explain the differences observed between taxa and may be the results of vicariant speciation. Xie et al. (2009) indicated that *Cincticostella* species were frequently collected in the recent years (meaning probably during the first decade of this century). However, they did not indicate where in China *C. femorata* was found and how widespread the species is. It will be of first interest from a biogeographic point of view to properly identify this material. This will allow the clarification of the distribution of *C. femorata* in China and may also clarify the distribution of *C. tornata*. It is also not excluded that collected specimens mentioned by Xie et al. (2009) partly belong to *C. tornata* or even to a still undescribed species.

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